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THE EARLY CLEAVAGE AND FORMATION OF THE MESODERM OF SERPULORBIS SQUAMIGERUS CARPENTER.

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THE material upon which the present paper is based was collected at San Pèdro, Cal., in the summer of 1895. Work upon it was carried on for a time during the winter of 1895–96, under Prof. C. O. Whitman, at the University of Chicago; but as the series of stages the material afforded proved incomplete, the subject was laid aside in the hope that, at some future time, when new material could be collected, the gaps might be filled. Since it is improbable that an opportunity of remedying this defect will soon present itself, and as the development of this form shows several interesting points of comparison as regards the formation of the mesoderm with what has recently been found to obtain in other mollusks and certain annelids, it was thought best to publish the present account. The development of Vermetus, a genus from which Serpulorbis is somewhat doubtfully distinct, has been studied by Salensky[1] in considerable detail. According to Salensky, mesoblastic pole cells do not appêar, and the mesoderm in Vermetus arises at a comparatively late period of development by a proliferation from the ectoderm in the region of the blastopore. With this conclusion my own observations do not agree, as certain stages that were found afford very clear evidence that the mesoderm arises

[1] *Archives de Biologie.* I, vi, 1887.

from the posterior macromere *D*, as has been found in so many other cases among mollusks and annelids.

Serpulorbis squamigerus is a common mollusk on the coast of southern California. The shell early loses all traces of its originally spiral form, and becomes bent and twisted in a very irregular way. Many individuals are often found tangled together, resembling a group of worm tubes, and forming masses of considerable size. The eggs are deposited in elongated capsules attached by one end a short distance within the mouth of the shell. In addition to the eggs the capsules contain numerous small cells, probably follicular, which doubtless serve to nourish the developing embryos. A large number of the eggs in each capsule fail to develop normally, and sooner or later break up into masses of isolated blastomeres. The cleavage of such eggs is irregular, sometimes from the start, but often the irregularity appears only after the egg has developed for some time in an apparently normal manner. As this departure from the typical path of development occurs at different stages in different eggs, it is not always easy to distinguish the normal from the abnormal process of cleavage.

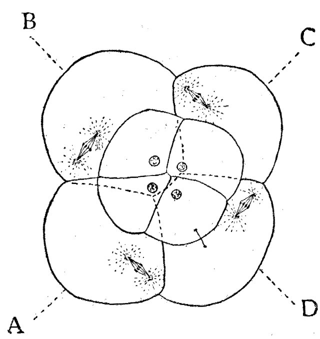

FIG. 1. — Eight-cell stage, seen from the animal pole. The dexiotropic origin of the first quartette of micromeres is indicated, and the spindles in the angles of the macromeres show that the next division will be laeotropic.

The first two cleavages are total and equal, giving rise to a four-cell stage of the usual molluscan type, in which two cells meet in a cross furrow at the vegetal pole. The next cleavage, which results in the formation of the first generation, or quartette, of micromeres, is dexiotropic. The micromeres are rather small, as is the rule in molluscan eggs, in which, as in the present case, there is a large amount of yolk. At the next cleavage the second quartette of micromeres are given off from the macromeres in a laeotropic direction. The spindles appear at one angle of the macromeres, but before the next division the nuclei wander through the cell so that the spindles next

appear at the opposite angle. The same migration is repeated in an opposite direction in preparation for the next ensuing division. The appearance of the second quartette is soon followed by a laeotropic division of the cells of the first. A dexiotropic cleavage of the second quartette next appears, and at about the same time the macromeres bud off the third quartette in a right-handed spiral, completing the separation of the ectoderm from the entoderm. The twenty micromeres composing the ectoderm are all transparent and devoid of yolk. While they form about one-half the surface of the egg, they

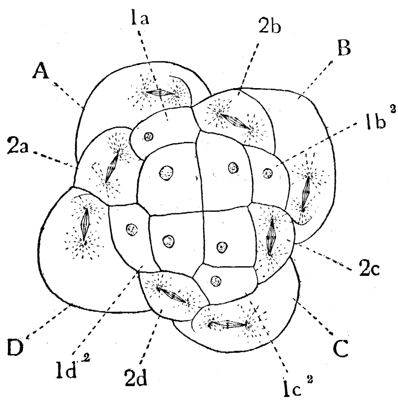

FIG. 2.

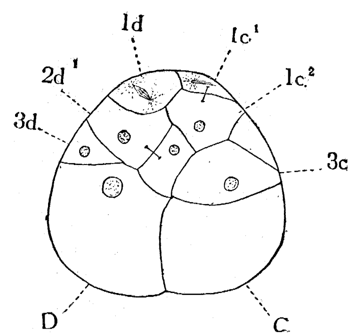

FIG. 3.

FIG. 2. — Sixteen to twenty-four cell stage from the animal pole, showing the origin of the third quartette and the dexiotropic cleavage of the second. The first quartette has divided, producing the four "trochoblasts."

FIG. 3. — Lateral view of the twenty-four cell stage. A cleavage is taking place in the apical cells.

form much less than half its bulk, as their thickness is not nearly so great as that of the large yolk-laden entomeres. The conclusion drawn by Salensky, that in Vermetus there are four quartettes of micromeres produced, is doubtless erroneous. The cleavage of the first quartette of ectomeres was probably overlooked, and the outer products of this division regarded as having had a separate origin from the macromeres. A comparison of Salensky's figures with the cleavage of Serpulorbis renders this interpretation probable. Besides, there are strong reasons for doubting that four generations of ectomeres are ever produced among the gasteropods, as I have attempted to show elsewhere.

The next cleavage occurs in the macromere *D*, and results in the formation of a yolk-laden cell, lying obliquely above the larger stem cell in such a way as to indicate that the division was laeotropic. This cell corresponds exactly as regards its time and mode of origin with the primary mesoblast cell of other mollusks. The corresponding division of the other three macromeres to form the remainder of the fourth quartette does not occur until a considerably later period. These divisions do not give rise to ectomeres, but to large yolk-laden entomeres, the cells of the fourth quartette being somewhat larger, if anything, than those at the vegetal pole.

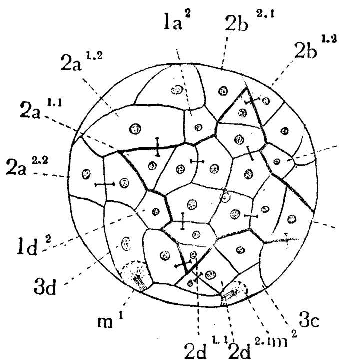

FIG. 4. — Forty-eight-cell stage, seen from the animal pole. The outline of the cross is marked with a heavier line. A dexiotropic twist is apparent in the arms of the cross. The small mesoblast cells are shown in dotted lines on the posterior side of the egg.

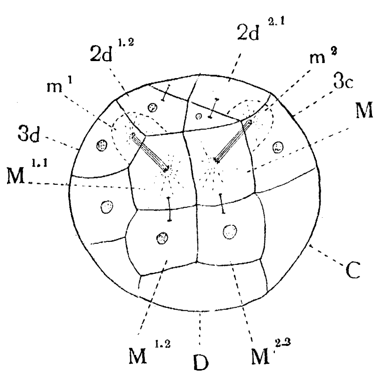

FIG. 5. — Posterior side of the same egg, showing the four derivatives of $4d$, the upper pair budding off the mesomeres, m^1 and m^2, into the interior of the egg.

About the time the primary mesoblast cell is given off the four apical cells of the first quartette divide in a dexiotropic direction, the outer products of the division forming the basal cells of the arms of the cross. Up to this time the cleavages of Serpulorbis agree, point for point, with those of Crepidula, Lymnaea, Limax, Planorbis, and Physa, with the exception that the divisions in the latter two genera are reversed. A comparison of the forty-eight-cell stage, shown in Figs. 4 and 5, indicates that the following divisions have taken place: The four upper cells of the second quartette have divided in a laeotropic direction, giving rise to the cells $2a^{1.1}$, $2b^{1.1}$, etc., which

form the tip cells of the arms of the cross. The four tip cells are smaller than the others, as in Crepidula and Planorbis. A cleavage of the four lower cells of the second quartette has taken place, likewise in a laeotropic direction. The second quartette now contains sixteen cells in four groups of four cells each. These cells bear exactly the same relations to each other, to the arms of the cross, and to the adjacent cells of the other quartettes, that they do in Crepidula, Planorbis, and several other forms at the corresponding stage of development. There can be no doubt, therefore, of their derivation, though their actual divisions were not all observed. The large entomeres, *A*, *B*, and *C*, have divided laeotropically, completing the formation of the fourth quartette. In place of the mesoblast cell 4 *D* there is a group of four cells, an upper pair containing little yolk, and a lower pair of about the same size in which the yolk is abundant. The origin of these cells was not followed, but there can be little doubt that they all owe their origin to the mesoblastic pole cell. They occupy the same area that was occupied by the pole cell. The cap of ectodermic cells is radially symmetrical, and contains no cells of sufficient size to have given rise to such large cells as the upper pair of the four without altering very materially the symmetrical relations shown in the figure. Besides, nothing corresponding to such a division is seen in other forms. In all probability these cells arose first by a horizontal division of the mesoblast cell, such as occurs in a large number of forms, and then by a division of the two daughter-cells in a plane at right angles to the previous one. At this period the egg contains a regular cap of ectodermic cells, four entomeres at the vegetal pole, three entomeres, 4 *a*, 4 *b*, and 4 *c* of the fourth quartette, and the group of four cells above described, in place of the remaining cell of the fourth quartette, 4 *d*. A comparison with the corresponding stage in the egg of Crepidula, as shown in Fig. 31 of Conklin's paper,[1] shows that the cells of the ectodermic cap correspond point for point, and also *that the four cells we have derived from 4 d are represented in Crepidula by four cells of subequal size having the same origin and position.* The fourth quartette is formed a

[1] *Journ. Morph.* Vol. xiii. 1897.

little later in Crepidula than the stage shown in Fig. 31, otherwise the two eggs are practically identical. The four cells in Serpulorbis are all on the surface of the egg and are not overlapped so much by the ectomeres as in Crepidula. The upper pair in Fig. 4 is shown in process of division. Each cell buds off a small cell into the interior of the egg, the spindles diverging anteriorly. At about the same period an almost exactly similar division occurs in Crepidula, the upper pair of cells budding off a small cell into the interior of the egg in very nearly the same direction. At a somewhat later stage in Serpulorbis, shown in Fig. 6, I have found two pairs of small cells lying entirely within the cleavage cavity which probably represent the descendants of the single pair whose origin has just been described. The parallelism with Crepidula extends also to this division as the corresponding pair of small cells in that form divides at about the same period. This is as far as the cleavage of these cells was carried. These results were worked out before Conklin's paper appeared, and as I did not follow the further history of these cells, as Conklin has succeeded in doing in Crepidula, it seemed uncertain what interpretation of them should be made. It seemed improbable that all of 4 *d* should form the mesoblast, as it was supposed to do in several forms. The four large cells showed no signs of passing into the interior of the egg, and it is probable that, after the mesoderm is separated from the upper pair, they enter into the formation of the entoderm, as in Crepidula.

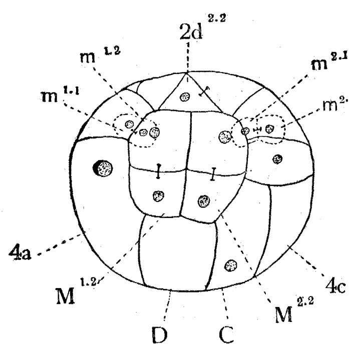

FIG. 6.—View of the posterior side of an egg in a somewhat later stage. There are seen two pairs of mesomeres in the cleavage cavity.

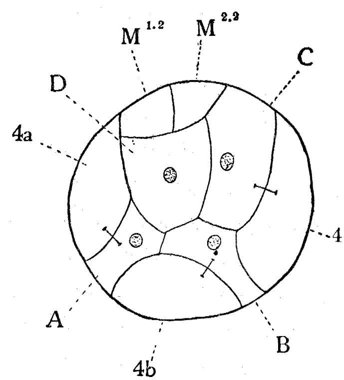

FIG. 7.—Vegetal pole of the same egg shown in Fig. 5.

Recent researches render it probable that the cell 4 *d* is not typically a purely mesoblastic cell. As Conklin has pointed out, the divisions of 4 *d* in Umbrella are strikingly like those in Crepidula, and strongly indicate, as Conklin maintains, that this cell contains both mesoderm and entoderm. The same cell in Cyclas was held by Stauffacher[1] to produce both mesoderm and entoderm. In Unio, Lillie[2] found that the pole cells budded off small cells at the surface before giving rise to the mesoblastic bands, and among annelids similar phenomena have been observed by Mead[3] in Amphitrite, by Wilson[4] in Nereis and Aricia, and by Treadwell[5] in Podarke. In Planorbis[6] I have found that a minute cell is budded off from each of the pole cells before they divide to form the mesoblastic bands. It has been suggested by Wilson that these minute cells correspond to the small entomeres found in Nereis, but they are budded off in a different direction and lie in the cleavage cavity instead of on the surface. This does not prove, however, that they are not homologous with entodermic cells, as a slight change in the direction of division of the pole cells would bring them in the wall of the blastula. And as they are probably not functional they may represent the last vestige of the entodermic portion of the mesentomeres.

[1] *Jen. Zeit.* Bd. xxviii. 1893.
[2] *Journ. Morph.* Vol. x. 1895.
[3] *Journ. Morph.* Vol. xiii. 1897.
[4] *Ann. N. Y. Acad. Sci.* Vol. xi. 1898.
[5] *Biological Lectures*, delivered at Woods Holl, Session of 1898, 1899.
[6] *Zoöl. Bull.* Vol. i. 1897.